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TI .omega.-Amide and .omega.-amino acid derivatives of .alpha.-ketoglutaric acid and oxalacetic acids
AU Otani, Theodore T.; Meister, Alton
CS Natl. Cancer Inst., Bethesda, MD
SO J. Biol. Chem. (1957), 224, 137-48

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TI The mechanism and specificity of the glutamine-.alpha.-keto acid transamination-deamidation
AU Meister, Alton
CS Natl. Cancer Inst., Bethesda, MD
SO J. Biol. Chem. (1954), 210, 17-35

Preparation and enzymic reactions of the keto analogs of asparagine and glutamine
AU Meister, Alton
CS Natl. Inst. of Health, Bethesda, MD
SO J. Biol. Chem. (1953), 200, 571-89
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ω-AMIDE AND ω-AMINO ACID DERIVATIVES OF α-KETOGLUTARIC AND OXALACETIC ACIDS*

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Recent studies in this laboratory indicate that α-ketoglutaramic and α-ketosuccinamic acids are intermediates in the enzymatic transamination-deamidation reactions of glutamine and asparagine, respectively (2, 3). The enzymatic synthesis of L-asparagine by transamination of α-ketosuccinamic acid with a number of L-α-amino acids has also been shown (4). The present report describes the preparation and study of several compounds related to α-ketoglutaramic and α-ketosuccinamic acids; these include the α-keto acid analogues of γ-glutamyl and β-aspartyl peptides. Compounds of this type have been found to be capable of existing in two interconvertible forms, only one of which exhibits properties characteristic of α-keto acids. β-Oxalacetylglycine and β-oxalacetylalanine, which exist predominantly in the reactive α-keto acid form, have been found to be active in enzymatic transamination to yield the corresponding β-aspartyl peptides. Studies on the unreactive forms of α-ketoglutaramic acid and related compounds are also described.

EXPERIMENTAL

α-Ketodicarboxylic Acid-ω-Amino Acids and Related Compounds—The preparation of α-keto acids by enzymatic oxidative deamination of the corresponding α-amino compounds has proved useful for a number of α-keto acids (5). Application of this procedure obviously depends on the susceptibility of the appropriate α-amino acid derivative to oxidation by the enzyme. Thus, the preparation of amide-substituted derivatives of α-ketoglutaramic and α-ketosuccinamic acids became possible when it was observed that certain γ-glutamyl and β-aspartyl amino acids were active substrates for snake venom L-amino acid oxidase (Table I). This study of the susceptibility of several β-aspartyl and γ-glutamyl derivatives revealed that γ-L-glutamyl-L-alanine, γ-L-glutamylglycine, γ-L-glutamyl-β-alanine,

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β-L-aspartylglycine, and β-L-aspartyl-L-alanine were oxidized. It is of interest, however, that γ-L-glutamyl-L-leucine was not oxidized.

TABLE I

*Relative Rates of Enzymatic Oxidation of α-Amino Dicarboxylic Acids and Their Derivatives**

Compound	Relative rate of oxidation
α-Aminomalonic acid	0
dl-α-Aminomalonamic acid	0
L-Aspartic acid	0
L-Aspartic acid-β-ethyl ester	106
L-Asparagine	83
β-L-Aspartylglycine	44
β-L-Aspartyl-L-alanine†	4
L-Glutamic acid	1
L-Glutamic acid-γ-ethyl ester	159
L-Glutamine	100
γ-L-Glutamylmethylamide	109
γ-L-Glutamylethylamide	109
γ-L-Glutamyldimethylamide	104
γ-L-Glutamylhydroxamic acid	70
γ-L-Glutamylhydrazide	63
γ-L-Glutamylglycine	30
γ-L-Glutamyl-L-alanine†	30
γ-L-Glutamyl-β-alanine	92
γ-L-Glutamyl-L-leucine	0
L-Glutathione	9‡
L-Homoglutamine	135
DL-α-Amino-δ-*N*-methyladipamic acid	118
α-L-Aminoadipic acid-δ-ethyl ester	111

* The reaction mixtures contained 13 μmoles of the substrate, 5 units of crystalline beef liver catalase (Worthington Biochemical Corporation), 20 mg. of rattlesnake venom, in a final volume of 1.3 ml. of 0.2 M potassium phosphate buffer, pH 7.5. The reaction was carried out at 37°; the gas phase was air. The relative rates of oxidation are expressed in terms of the rate for L-glutamine, which is arbitrarily assigned a value of 100. The rate for L-glutamine was 9.20 μl. per minute.

† These products also contained the analogous α isomers as indicated in the text. In these experiments the concentration of the ω isomer was 0.01 M.

‡ Based on ammonia formation.

The γ-glutamyl and β-aspartyl peptides were prepared via the corresponding *N*-carbobenzoxy-ω-amino acid azides (6). Two of the products, γ-L-glutamyl-L-alanine and β-L-aspartyl-L-alanine, were found to contain appreciable quantities of the isomeric α-peptides. Our experience is thus similar to that of Sachs and Brand (7), who obtained mixtures of α- and γ-glutamyl peptides by this procedure. With the exception of these two

products, the ω-peptide preparations used in this study gave equimolar quantities of carbon dioxide when treated with ninhydrin according to the Van Slyke gasometric procedure (8), and they were homogeneous on paper chromatography.

The general procedure for preparation of α-keto acids by enzymatic oxidation of the analogous α-amino acids was followed (5). Relatively large quantities of enzyme were used in order to complete the oxidation in a short period, thereby reducing losses due to spontaneous breakdown of the products; an excess of dialyzed crystalline beef liver catalase (Worthington Biochemical Corporation) was added.

Barium γ-(α-Ketoglutaryl)glycinate[1]—The reaction mixture consisted of 500 mg. of γ-L-glutamylglycine, catalase (100 units), and 1.0 gm. of rattlesnake (*Crotalus adamanteus*) venom in a final volume of 20 ml. After 10 hours, the reaction reached 78 per cent of theoretical completion, and the product was processed as described (5). The yield of the barium salt was 509 mg. (60 per cent). Calculated[2] for $C_7H_7O_6NBa \cdot \frac{1}{2}H_2O$. C 24.2, H 2.3, N 4.0, Ba 39.6; found, C 24.1, H 2.5, N 3.9, Ba 39.9.

Barium γ-(α-Ketoglutaryl)-β-alaninate—200 mg. of γ-L-glutamyl-β-alanine were oxidized with 769 mg. of venom[3] in a volume of 50 ml. The reaction was 94 per cent complete after 4.5 hours. The yield was 213 mg. (65 per cent). Calculated for $C_8H_9O_6NBa \cdot \frac{1}{2}H_2O$. C 26.6, H 2.8, N 3.9, Ba 38.0; found, C 26.6, H 3.2, N 3.7, Ba 38.4.

Barium γ-(α-Ketoglutaryl)-L-alaninate—The oxidation was carried out with 3.0 gm. of L-glutamyl-L-alanine (64 per cent γ isomer) and 4 gm. of venom[3] in a volume of 57 ml. After 8 hours, oxidation of the γ isomer was 83 per cent complete; the yield was 1.29 gm. (41 per cent, based on the γ isomer). Calculated for $C_8H_9O_6NBa \cdot \frac{1}{2}H_2O$. C 26.6, H 2.8, N 3.9, Ba 38.0; found, C 26.6, H 2.8, N 3.9, Ba 38.0.

Barium β-Oxalacetylglycinate—Oxidation of 374 mg. of β-L-aspartylglycine was carried out with 1.38 gm. of venom[3] in a volume of 40 ml. The reaction proceeded to 85 per cent of completion after 6 hours; the yield was 480 mg. (73 per cent). Calculated for $C_6H_5O_6NBa \cdot \frac{1}{2}H_2O$. C 21.6, H 1.8, N 4.2, Ba 41.2; found, C 21.4, H 2.2, N 4.1, Ba 41.3.

Barium β-Oxalacetyl-L-alaninate—1.2 gm. of L-aspartyl-L-alanine (50 per cent β isomer) were oxidized with 1.9 gm. of venom[3] in a volume of 50

[1] In this paper γ-(α-ketoglutaryl)glycine, γ-(α-ketoglutaryl)-L-alanine, γ-(α-ketoglutaryl)-β-alanine, β-oxalacetylglycine, and β-oxalacetyl-L-alanine, respectively, refer to *N*-(carboxymethyl)-2-oxoglutaramic acid, *N*-(1-carboxyethyl)-2-oxoglutaramic acid, *N*-(2-carboxyethyl)-2-oxoglutaramic acid, *N*-(carboxymethyl)-2-oxosuccinamic acid, and *N*-(1-carboxyethyl)-2-oxosuccinamic acid.

[2] The microanalyses reported here were performed by Mr. Robert J. Koegel and by Dr. William C. Alford and their staffs.

[3] 100 units of catalase were added.

ml. After 7.5 hours, the reaction was terminated when 41 per cent of the susceptible peptide was oxidized. The yield was 300 mg. (29 per cent, based on the β isomer). Calculated for $C_7H_7O_6NBa \cdot H_2O$. C 23.6, H 2.5, N 3.9, Ba 38.6; found, C 23.6, H 2.6, N 3.7, Ba 38.6.

The α-keto acid-ω-amino acid derivatives were treated with ceric sulfate-sulfuric acid and with hydrogen peroxide at pH 4.9, under conditions similar to those previously employed (5). As indicated in Table II, all of the products were decarboxylated by ceric sulfate-sulfuric acid. On the other hand, the γ-(α-ketoglutaryl)amino acids, like α-ketoglutaramic acid, did not yield carbon dioxide when treated with hydrogen peroxide, a finding consistent with the occurrence of these compounds in an unreactive form.

TABLE II

Decarboxylation of α-Ketodicarboxylic Acid-ω-Amino Acids

Compound	Per cent decarboxylation with ceric sulfate*	Per cent decarboxylation with H_2O_2†
γ-(α-Ketoglutaryl)glycine	103	0
γ-(α-Ketoglutaryl)-β-alanine	106	0
γ-(α-Ketoglutaryl)-L-alanine	102	0
β-Oxalacetylglycine	103	81
β-Oxalacetyl-L-alanine	109	86
Pyruvic acid	100	100

* The reactions were carried out in Warburg vessels at 37°. The main compartment contained 5 μmoles of keto acid in 0.5 ml. of 0.2 N HCl, and the side bulb contained 0.3 ml. of ceric sulfate (0.2 M in 2 N H_2SO_4).

† The reactions were carried out in Warburg vessels at 37°. The main compartment contained 5 μmoles of keto acid in 0.5 ml. of 0.2 M sodium acetate buffer at pH 4.9, and the side bulb contained 0.3 ml. of M hydrogen peroxide.

The β-oxalacetylamino acids were decarboxylated to a considerable extent by peroxide, suggesting that they were predominantly in the reactive open chain form. It is of interest that α-ketoglutaramic acid exhibits a much greater tendency to exist in the unreactive form than does α-ketosuccinamic acid (2); the analogous amino acid derivatives thus exhibit similar behavior. Ultraviolet absorption curves of these compounds in alkali are described in Fig. 1. α-Ketosuccinamic acid and its derivatives, the β-oxalacetyl-ω-amino acids, absorb strongly in the region of 290 mμ, and α-ketoglutaramic acid and its derivatives, the α-keto-γ-glutaryl-ω-amino acids, show no significant peaks between 220 and 400 mμ. The high characteristic absorption of the β-oxalacetyl derivatives in alkali may be ascribed to the enolic forms of these compounds (2).

Peroxide oxidation of the *reactive* forms of α-ketoglutaramic and α-ketosuccinamic acids would be expected to yield succinamic and malonamic acids, respectively, and treatment of γ-(α-ketoglutaryl)alanine with hydro-

gen peroxide should yield succinylalanine. A 5 mmole sample of γ-(α-ketoglutaryl)-L-alanine was mixed with 0.25 ml. of M hydrogen peroxide in 1.0 N sodium hydroxide, and, after standing for 5 minutes at room temperature, the solution was acidified with concentrated hydrochloric acid and

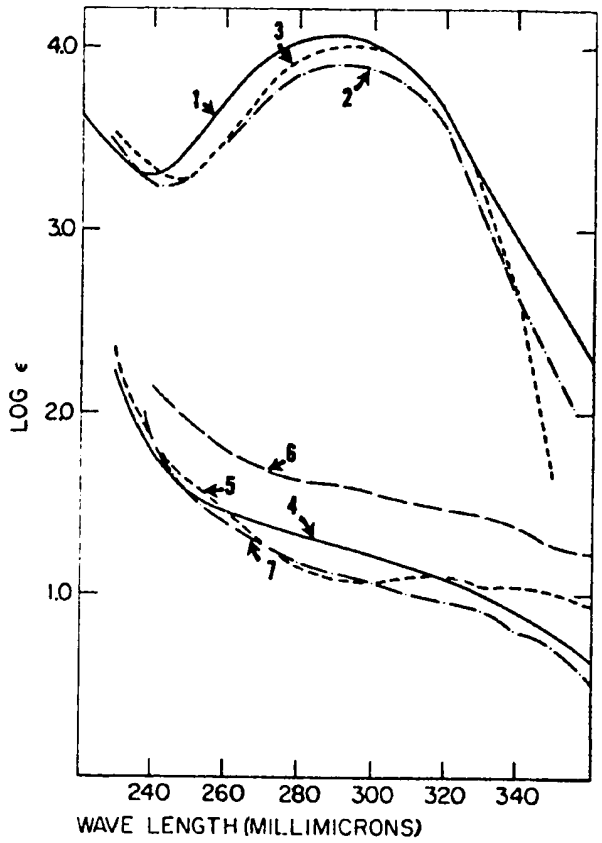


FIG. 1. Ultraviolet absorption curves of several α-keto acid-ω-amides in 0.1 N NaOH. The absorption curves were carried out with a Cary model No. 14 recording spectrophotometer. Curve 1, α-ketosuccinamic acid (6.20×10^{-5} M); Curve 2, β-oxalacetylglycine (6.21×10^{-5} M); Curve 3, β-oxalacetyl-L-alanine (6.45×10^{-5} M); Curve 4, α-ketoglutaramic acid (3.24×10^{-3} M); Curve 5, γ-(α-ketoglutaryl)glycine (3.19×10^{-3} M); Curve 6, γ-(α-ketoglutaryl)-L-alanine (3.34×10^{-3} M); Curve 7, γ-(α-ketoglutaryl)-β-alanine (3.31×10^{-3} M). The ordinate represents the logarithm of the molar extinction.

placed at 100° for 1 hour. The cooled solution was chromatographed on Whatman No. 4 paper with four solvent systems.[4] Alanine was demonstrated by ninhydrin treatment of the chromatograms, and succinic acid was located and identified by the method of Rydon and Smith (9), modified

[4] The following solvents were used: (*a*) liquefied phenol saturated with 10 per cent sodium citrate; (*b*) formic acid, 15 parts, *tert*-butanol, 70 parts, and water, 15 parts; (*c*) pyridine, 4 parts, methanol, 80 parts, water, 20 parts; (*d*) *n*-butanol, 200 parts, glacial acetic acid, 30 parts, and water, 75 parts.

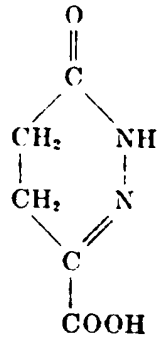
as described (10). Similar studies with β-oxalacetylglycine yielded a spot identical in four solvents with that of glycine. Malonic acid could not be detected; however, an authentic sample of malonic acid was destroyed under these conditions.

In addition to providing evidence for the structure of the α-keto acid-ω-amides, the hydrogen peroxide procedure has been used for the preparation of the decarboxylation products. Thus, succinamic and malonamic acids were prepared by decarboxylation of the appropriate α-keto acid-ω-amides. They were also prepared by enzymatic oxidation in the absence of catalase.

Succinamic Acid—1.35 gm. of barium α-ketoglutaramate were dissolved in a mixture of 5 ml. of 0.2 M sodium hydroxide and 5 ml. of 30 per cent hydrogen peroxide. After standing at room temperature for 4 hours, the excess peroxide was destroyed by addition of crystalline beef liver catalase, and the solution was added to the top of a Dowex 50 column (50 × 2.5 cm.) in the acid form. The product was eluted with water. Succinamic acid was obtained by evaporation of the effluent *in vacuo* and crystallized from ethanol. The yield was 500 mg. or 67 per cent. Calculated for $C_4H_7O_3N$. C 41.0, H 6.0, N 12.0; found, C 40.8, H 6.1, N 11.8.

Sodium Malonamate—3.626 gm. of L-asparagine monohydrate were oxidatively deaminated with 1.2 gm. of *C. adamanteus* venom as described previously (5), except that catalase was omitted. The sodium salt of malonamic acid was isolated as described for sodium α-ketosuccinamate. The yield was 2.4 gm. or 80 per cent. Calculated for $C_3H_4O_3NNa$. C 28.8, H 3.2, N 11.2, Na 18.4; found, C 28.7, H 3.6, N 11.0, Na 18.2.

Enzymatic Oxidation of γ-L-Glutamylhydrazide—Oxidative deamination of 1 gm. of γ-L-glutamylhydrazide was carried out with 1 gm. of venom in the presence of excess catalase[3] in a volume of 40 ml. The reaction was 93 per cent complete after 8 hours, and the product was isolated as described (5) as the free acid. The yield was 510 mg. (58 per cent). The product did not form a 2,4-dinitrophenylhydrazone, nor was it decarboxylated by hydrogen peroxide. On treatment with ceric sulfate, there was only a very slow evolution of gas. The properties and elemental analyses were consistent with the formation of the internal hydrazone, pyridazinone-3-carboxylic acid.



Calculated for $C_5H_6O_3N_2$ C 42.3, H 4.3, N 19.7; found, C 42.3, H 4.4, N 19.8. The melting point of this compound was observed to be 199°; a melting point of 198° was reported for pyridazinone-3-carboxylic acid prepared by an independent procedure (11).

Enzymatic Transamination—It was found that β-oxalacetylglycine and β-oxalacetyl-L-alanine were capable of transamination in the glutamine transaminase-deamidase system (12, 13) to form β-aspartylglycine and β-aspartylalanine, respectively (Table III). The latter compounds were identified by paper chromatography[4] and were quantitatively determined by densitometric measurements (14). There was no observable transamination when glutamine was replaced by glutamate with the purified glutamine system or with a crude rat liver preparation. Neither the β-oxalacetylamino acids nor the β-aspartylamino acids were hydrolyzed by these enzyme preparations. In contrast to the β-oxalacetyl derivatives, the γ-(α-ketoglutaryl)amino acids were not active in transamination with glutamine or glutamic acid under these conditions.

TABLE III

*Enzymatic Transamination Studies**

Substrate	Glutamine disappearance	Peptide formation	NH_3 liberated
	μmoles	*μmoles*	*μmoles*
β-Oxalacetylglycine	4.7	4.9†	4.2
β-Oxalacetyl-L-alanine	2.0	1.7‡	1.9
γ-(α-Ketoglutaryl)glycine	0	0	0
γ-(α-Ketoglutaryl)-L-alanine	0	0	0
α-Ketoisocaproic acid			6.1§

* The reaction mixtures contained initially 10 μmoles of α-keto acid, 10 μmoles of L-glutamine, and 20 mg. of glutamine transaminase preparation (12) in a final volume of 0.4 ml. of 0.04 M Veronal buffer of pH 7.9; incubated for 3 hours at 37°. Glutamine disappearance was determined as described (12).

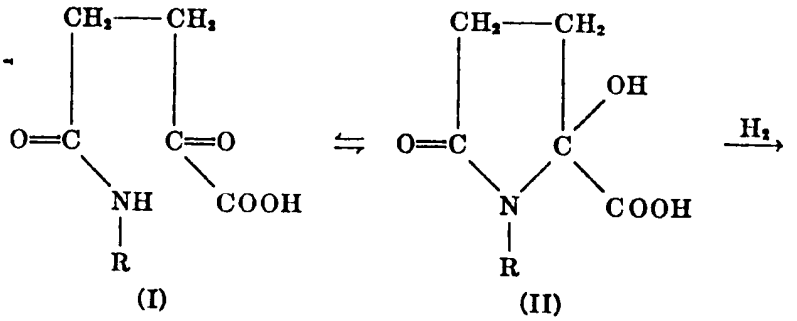
† β-Aspartylglycine.

‡ β-Aspartylalanine.

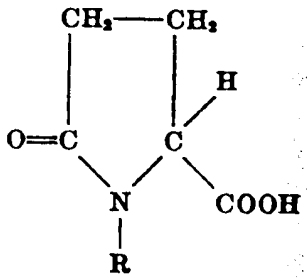
§ The formation of leucine was observed by paper chromatography.

Studies on Unreactive Forms of α-Ketodicarboxylic Acid-ω-Amides—Experiments involving the reaction of a series of α-keto acid-ω-amides with 2,4-dinitrophenylhydrazine, decarboxylation by hydrogen peroxide and by ceric sulfate, and susceptibility to lactic dehydrogenase and liver ω-amidase, indicated that formation of an unreactive form (previously designated form A (2)) is associated with a carbon chain length of 4 or 5 atoms and an ω-amide group, in which at least 1 hydrogen atom is unsubstituted (3). It was suggested earlier that the unreactive forms might represent cyclic or polymeric modifications of the α-keto acids (2). It was also

observed that both reactive and unreactive forms possess an acid group, presumably a carboxyl group, which has a pK value of 2.6. Evidence indicated that the two forms existed in equilibrium in solution and that alkali favored conversion of the unreactive to the reactive form. The chain length requirements suggest ring formation, and the necessity for a hydrogen atom on the ω-amide group suggests that ring closure might involve this group. Furthermore, the reduced rate of reaction with carbonyl reagents is consistent with a linkage involving the α-keto group. The possibility that enolization might be responsible for the anomalous behavior of these compounds was rendered improbable by enol titration carried out according to a modification of the Kurt Meyer procedure (15), which showed that α-ketoglutaramic acid, α-ketosuccinamic acid, and related compounds are not appreciably enolized except in alkaline solution (see Fig. 1, above). Cyclization by elimination of the elements of water between the ω-amide and carboxyl groups forming an imide appears unlikely in view of the presence of a strong titratable acid group. Cyclization of the open chain form of α-ketoglutaramic acid (I) by reaction between the α-keto and ω-amide groups to yield a ketolactam (II) is conceivable for α-ketoglutaramic acid and derivatives.



(I) (II)



(III)

Such a formulation is analogous to one proposed for the anilide and amide of levulinic acid (16). Consistent with the belief that Structure II (where R = H) represents the structure of the unreactive form of α-ketoglutaramic acid is the observation that catalytic hydrogenation of α-ketoglutaramic

acid gave pyrrolidonecarboxylic acid (Structure III). A 200 mg. sample of α-ketoglutaramic acid in 10 ml. of water and 400 mg. of platinum oxide catalyst were placed in a Parr hydrogenation apparatus at 26° and 40 pounds pressure for 48 hours. After hydrogenation and removal of the catalyst, the solution was evaporated *in vacuo* to dryness. Paper chromatography of the residual solid material in four solvents indicated conversion of approximately 80 per cent of the α-ketoglutaramic acid to pyrrolidonecarboxylic acid; the spots were rendered visible by the modified Rydon and Smith procedure (9, 10). Pyrrolidonecarboxylic acid (110 mg.; m.p. 183°; mixed m.p. 183°) was obtained after recrystallization from ethanol and ether.

A structure analogous to Structure II for the unreactive form of α-ketosuccinamic acid appears unlikely inasmuch as it would be necessary to postulate a four-membered ring. The possibility that the unreactive form of α-ketosuccinamic acid might be a polymer led us to request the assistance of Dr. Gerson Kegeles, who carried out three determinations of molecular weight by a sedimentation procedure.[5] The unreactive form of α-ketosuccinamic acid was sedimented as a neutral undissociated molecule in 0.1 N hydrochloric acid according to the method of Klainer and Kegeles (17). Values of 252, 260, and 268 were obtained, representing good agreement with the theoretical molecular weight of 262 for a dimer.[6] A determination of molecular weight carried out on a sample of the reactive form of α-ketosuccinamic acid, prepared by treatment of the unreactive form with sodium hydroxide followed by acidification with hydrochloric acid, gave a value of 130 (theoretical for the monomer = 131). This represents excellent evidence for the belief that the unreactive form of α-ketosuccinamic acid is a dimer, the structure of which is now under investigation.

Infrared absorption studies of α-ketoglutaramic acid, α-ketosuccinamic acid, and related compounds have been carried out (1); these data are consistent with the proposed structures, but do not appear to permit definite conclusions to be drawn.

DISCUSSION

The present findings suggest that the unreactive form of α-ketoglutaramic acid, and presumably also those of its *N*-substituted derivatives, is a cyclic compound involving interaction between the amide and α-carbonyl groups (II). Recently, Cohen and Witkop (18) have observed a reaction

[5] The authors are grateful to Dr. Gerson Kegeles for his kindness in performing these determinations.

[6] The determinations of molecular weight were based on an apparent specific volume of 0.506 determined by the authors for a solution containing 1.9743 gm. of dimer per 100 gm. of water.

of this type between amide nitrogen and carbonyl groups across a ten-membered ring to yield a ketolactam, the structure of which was proved by reduction to the corresponding saturated compound. It is also of interest that the formation of the ketolactam was spontaneous. The rapid, pH-dependent interconversion between the reactive and unreactive forms of α-ketoglutaramic acid (2) is consistent with a shift between the open chain and ketolactam forms. In contrast, the reactive form of α-ketosuccinamic acid is relatively stable; although the unreactive form of α-ketosuccinamic acid is rapidly converted to the open chain form in alkali, subsequent acidification does not produce prompt reversion to the unreactive form (2). These observations serve to distinguish the unreactive form of α-ketosuccinamic acid from that of α-ketoglutaramic acid, and are consistent with a monomer-dimer system for α-ketosuccinamic acid. The *N*-substituted derivatives of α-ketosuccinamic acid described here exhibit less tendency to yield unreactive forms than does α-ketosuccinamic acid. The significance of this observation must await work on the structure of the dimer.

The demonstration of enzymatic transamination of β-oxalacetylamino acids to the corresponding β-aspartylamino acids is of interest in that it extends the known scope of enzymatic transamination to reactions involving compounds which possess a peptide bond. However, there is as yet no conclusive evidence for the enzymatic transamination of α-peptides. The failure of the γ-(α-ketoglutaryl)amino acids to participate in transamination may probably be ascribed to the tendency of these compounds to exist predominantly in a cyclic form. Although the participation of γ-glutamyl peptides in transpeptidation has been amply demonstrated (19, 20), the significance of these reactions is not yet known. It is of interest that Hendler and Greenberg (21) observed formation of a compound with the properties of γ-(α-ketoglutaryl)glycine in mouse spleen and rat kidney systems containing γ-glutamylglycine. Further study of the metabolic role of γ-glutamyl peptides, β-aspartyl peptides, and their α-keto analogues is necessary.

SUMMARY

1. The oxidation of a series of α-L-aminodicarboxylic acids and derivatives by snake venom L-amino acid oxidase has been described; β-aspartylglycine, β-aspartylalanine, γ-glutamylalanine, γ-glutamyl-β-alanine, and several other compounds were oxidized, but γ-glutamylleucine was not attacked.

2. The α-keto analogues of several ω-peptides have been prepared, including those corresponding to γ-glutamylglycine, γ-glutamylalanine, β-aspartylglycine, and β-aspartylalanine. The product of the enzymatic

oxidative deamination of γ-L-glutamyl hydrazide (pyridazinone-3-carboxylic acid) was isolated. The enzymatic preparation of succinamic acid and sodium malonamate was described.

3. The β-oxalacetylamino acids (β-oxalacetylglycine and β-oxalacetylalanine) differed from the γ-(α-ketoglutaryl)amino acids and α-ketoglutaramic acid in being decarboxylated by hydrogen peroxide. It was therefore concluded that these β-oxalacetylamino acids exist predominantly in the reactive open chain form.

4. Under the conditions employed, the γ-(α-ketoglutaryl)amino acids did not participate in enzymatic transamination. On the other hand, β-oxalacetylglycine and β-oxalacetylalanine transaminated with glutamine to form, respectively, β-aspartylglycine and β-aspartylalanine.

5. The evidence suggests that α-ketoglutaramic acid, and presumably its *N*-substituted derivatives, exists predominantly in an unreactive ketolactam form, which is in equilibrium with the open chain α-keto acid form. The unreactive form of α-ketosuccinamic acid has been shown by the sedimentation method of Klainer and Kegeles to have a molecular weight consistent with that of a dimer.

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